

During the initial Grant Cycle (1998-2003) we have initiated twin studies of biologic marker assessments (this project) concomitant with pain diary studies (Project I) in infants and young children to identify in a longitudinal prospective manner any correlation between these surrogate markers and the onset of microvessel occlusion leading to pain. We chose this specific period of life (the first 4 to 5 years) since, during this time, HbF levels (which are initially high) will decrease, attaining levels commensurate with that observed in later life, i.e. a window of opportunity exists in which to seek relationships between biologic markers and HbF. Our publications to date have demonstrated important inverse correlations between F cells and adhesion; and also between F cells and red cell phosphatidylserine (PS) with consequent modulation of hemostatic activation. Prospective studies on 22 infants with SCD recruited  $\leq$  6 months of life in conjunction with the pain diary observations (Project I) were analyzed at our request by the NIH Statistical Center (Rho, Inc.). **Preliminary results suggest that as these infants are being followed, elevated functional adhesion ratios and elevated levels of red cell phosphatidylserine (PS) may predict the infant with the greater pain incidence.** Power calculations demonstrate that the required sample size necessary is a cohort of approximately 100 infants with SS or SC disease, for the successful completion of these studies. **This work combining hard science, i.e. biologic marker analyses with quantitative pain data is unique in the area of sickle cell research.** We have also broadened the scope of our marker studies to add quantitative and qualitative assessments of circulating endothelial cells (CECs), including phenotypic studies for tissue factor (TF) and P selectin, and a microparticle capture system assay that should provide additional new information on PS-positive endothelial, platelet, and white cell vesicles. Hemostatic markers will now include assays of whole blood and monocyte TF, as well as determinations of platelet TF (which in our preliminary studies was elevated – an observation not hitherto reported in this disease entity). Studies of white cell activation will include sol L selectin, elastase levels and FACS for CD64. Plasma levels of circulating ligands/receptors will include proadhesive sVCAM-1 and thrombospondin (TSP), and the potent angiogenic compound VEGF. The studies will provide critical information on the importance of specific biologic markers (including the new marker assessments) as they relate to the pathophysiology of microvascular occlusion. **The longitudinal studies will create a “biologic footprint” as the infant grows,** providing a unique look at the temporal sequence of changes in adherence, endothelial, platelet, white cell and hemostatic activation in infants as the protective effects of HbF decline, and the subject begins to “cope” with the unfolding systemic effects of HbS polymerization.